REMARKS

Accompanying this response, please find marked-up paragraphs of the specification which overcome some informalities noted in the specification. The specification amendments were entered during Chapter II examination of the parent PCT patent application. The specification amendments were made to overcome formal objections raised in the written opinion dated October 23, 2001. The undersigned avers that the enclosed replacement paragraph(s) of the specification corresponds to the Chapter II PCT amendments and do not contain any new matter.

In the event that there are any fee deficiencies or additional fees are payable, please charge the same or credit any overpayment to our Deposit Account (Account No. 04-0213).

Respectfully submitted,

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By: _____

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[013] DE 199 01 761 describes a method of electrochemically detecting sequence specific nucleic acid oligomer hybridization events, wherein the difference in conductivity of single-strand nucleic acid oligomers and double-strand nucleic acid oligomers is the key criteria for detecting a hybridization event. Given a suitable arrangement of the nucleic acid oligomer as part of an electrochemical cell, the conductivity difference can be used as a "molecular switch" within an otherwise closed electrical circuit. In the non-hybridized state (as a single strand), the nucleic acid oligomer acts as an open switch, and upon hybridization with the complementary counter strand, the switch is closed.

[014] According to DE 199 01 761, the probe oligonucleotides of the individual test sites (dots with probe oligonucleotides having identical sequences) are immobilized on an electrode, and a "redox moiety" is covalently attached at the free end of the probe oligonucleotides as an electromarker.

[015] The redox moiety (electromarker), a complex comprising the electron donor "D" and electron acceptor (A," is externally stimulated by light to charge separation. In this process, an electron is transferred from the donor to the acceptor and a charge-separated state D^+A^- is formed. The conductivity of the hybridized probe oligonucleotide is high, i.e. given a suitable electrode potential, an electron can be transferred from A^- to the electrode and detected. In the presence of a soluble reducing agent X^- that reduces D^+ to D, the redox moiety is set in a pre-light-absorption state. Additional light absorption starts the cycle anew and produces a distinct current. The conductivity of the probe oligonucleotide without a complementary target, on the other hand, is low, the electron cannot be transferred from A^- to the electrode, the cycle is interrupted, and no current flows.

[016] Thus, although quantitative and extremely sensitive methods for DNA/RNA sequencing exist, these methods are time consuming, require painstaking sample

preparation and expensive equipment, and are generally not available as portable systems.

[017] DESCRIPTION OF THE INVENTION

[018] Therefore, it is the object of the present invention to create for detecting nucleic acid oligomer hybrids an apparatus and a method that do not exhibit the disadvantages of the background art.

[019] According to the present invention, this object is solved by the modified nucleic acid oligomer according to independent claim 1~~, the method of producing a modified nucleic acid oligomer according to independent claim 22, the modified conductive surface according to independent claim 34, the method of producing a modified conductive surface according to independent claim 49, and a method of electrochemically detecting nucleic acid oligomer hybridization events according to independent claim 53~~.

[020] The following abbreviations and terms will be used in the context of the present invention:

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PNA	Peptide nucleic acid (synthetic DNA or RNA in which the sugar-phosphate moiety is replaced by an amino acid. If the sugar-phosphate moiety is replaced by the -NH-$(CH_2)_2$-N($COCH_2$-base)-CH_2CO- moiety, PNA will hybridize with DANN.)
A	Adenine
G	Guanine
C	Cytosine
T	Thymine
U	uracil
base	A, G, T, C, or U
bp	base pair
nucleic acid	At least two covalently-joined nucleotides or at least two

	covalently-joined pyrimidine (e.g. cytosine, thymine, or uracil) or purine bases (e.g. adenine or guanine). The term nucleic acid refers to any backbone of the covalently-linked pyrimidine or purine bases, such as the sugar-phosphate backbone of DNA, cDNA, or RNA, a peptide backbone of PNA, or analogous structures (e.g. a phosphoramide, thiophosphate, or dithiophosphate backbone). The essential feature of a nucleic acid according to the present invention is that it can sequence-specifically bind naturally occurring cDNA or RNA.
nucleic acid oligomer	Nucleic acid of a base length that is not further specified (e.g. nucleic acid octamer: a nucleic acid having any backbone in which 8 pyrimidine or purine bases are covalently bound to one another).
oligomer	Equivalent to nucleic acid oligomer.
oligonucleotide	Equivalent to oligomer or nucleic acid oligomer, e.g. a DNA, PNA, or RNA fragment of a base length that is not further specified.
oligo	Abbreviation for oligonucleotide.
primer	Initial complementary fragment of an oligonucleotide, the base length of the primer being only approx. 4-8 bases. Serves as the starting point for enzymatic replication of the oligonucleotide.
mismatch	To form the Watson-Crick double-stranded oligonucleotide structure, the two single-strands hybridize in such a way that the A (or C) base of one strand forms hydrogen bonds with the T (or G) base of the other strand (in RNA, T is replaced by uracil). Any other base pairing does not form hydrogen bonds, distorts the structure and is referred to as a "mismatch".
ss	single-strand
ds	double-strand
redox-active moiety	Equivalent to a catalytically redox-active moiety.
catalytically redox-	In the context of the present invention, a moiety referred to using

active moiety

the generic term "catalytically redox-active moiety" usually consists of one or more redox-active centers (cofactors, prosthetic groups), which are referred to in the following as electron donors or electron acceptors, and one or more macromolecules binding these redox-active centers. Thus, in its form that is relevant to the present invention, the catalytically redox-active moiety includes one or more electron-donor molecules and/or one or more electron-acceptor molecules, this (these) electron-donor molecule(s) and/or this (these) electron-acceptor molecule(s) being/becoming bound to one or more macromolecules or being embedded in this (these) macromolecule(s). Electron donor(s) and/or electron-acceptor(s) may be joined with one another via one or more covalent or ionic bonds, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance, covalent links being able to be direct or indirect (e.g. via a spacer, but not via a nucleic acid oligomer) links. In addition, the electron donor(s) and/or electron acceptor(s) may be joined with the macromolecule(s) via covalent attachment to the macromolecule(s), via encapsulation in suitable molecular cavities (binding pockets) of the macromolecule(s), via ionic bonds, hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance between the macromolecule(s) and the electron-donor molecule(s) and/or electron-acceptor molecule(s). If the catalytically redox-active moiety is composed of multiple macromolecules, the binding of the macromolecules to one another may likewise take place covalently, ionically, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance. In the minimum case, a catalytically redox-active moiety may also consist of only one macromolecule,

	the macromolecule, in its form that is relevant to the present invention, then also acting as the electron donor or acceptor. It may also consist of only one electron donor or acceptor. In addition, the catalytically redox-active moiety may also be formed through spontaneous congregation of the components in solution (in situ). In addition to comprising electron donor(s) and/or electron acceptor(s) and macromolecule(s), essential features of the catalytically redox-active moiety are: (i) in the forms relevant to the present invention (electron donor(s) and/or electron acceptor(s) in their original state or in an oxidized or reduced state), the moiety is stable and does not dissociate into its components, (ii) the electrocatalytic activity of the moiety (see below), (iii) the moiety includes no nucleic acid, (iv) the moiety's composition comprising electron donor(s) and/or electron acceptor(s) and/or macromolecule(s) can be recognized by a person skilled in the art, regardless of the bond between the components, since, in principle, the redox-active centers (cofactors, prosthetic groups) and the affiliated matrix comprising macromolecule(s) (e.g. the apoprotein in the case of enzymes, as an example of a catalytically redox-active moiety) may also occur separately. The catalytically redox-active moiety may be for example any redox-active protein/enzyme from the group of oxidases or reductases; from this group of oxidases or reductases, proteins/enzymes modified by protein engineering or gene mutation; or an artificially produced moiety comprising one or more redox-active centers (electron donor or acceptor) or an artificially produced moiety comprising one or more redox-active centers (electron donor or acceptor) and one or more macromolecules binding these redox-active centers.
cofactor	Equivalent to a redox-active center (electron donor or acceptor) of the catalytically redox-active moiety.

prosthetic group	Equivalent to a redox-active center (electron donor or acceptor) of the catalytically redox-active moiety.
redox-active center of the catalytically redox-active moiety	The redox-active center of the catalytically redox-active moiety is characterized in that it acts as an electron donor or acceptor toward a substrate specific to the catalytically redox-active moiety. Moreover, if a catalytically redox-active moiety possesses multiple redox-active centers (electron donors and/or electron acceptors), a charge transfer may occur within the catalytically redox-active moiety: following the charge transfer between the substrate specific to the catalytically redox-active moiety and a first redox-active center, an additional charge transfer is possible between this first redox-active center and an additional redox-active center of the same catalytically redox-active moiety, this second redox-active center, in turn, being able to transfer charge to a third redox-active center, and so on. Thus, a successive charge transfer may occur within the catalytically redox-active moiety if the catalytically redox-active moiety includes multiple redox-active centers. In this case, the process of successive charge transfer is initiated by the presence of the substrate (with its property of spontaneously transferring a charge between the substrate and the catalytically redox-active moiety) specific to the catalytically redox-active moiety.
electron-donor molecule	Equivalent to an electron donor.
electron-acceptor molecule	Equivalent to an electron acceptor.
electron donor	In the context of the present invention, the term "electron donor" refers to a component of the catalytically redox-active moiety. An electron donor is a molecule that can transfer an electron to an electron acceptor, directly or under the influence of certain external conditions. For example, one such external condition is the oxidation or reduction of the electron donor or acceptor of the

catalytically redox-active moiety by an external oxidizing or reducing agent, so for example the transfer of an electron to the electron donor by a reducing agent, or the release of an electron by the electron acceptor to an oxidizing agent. These oxidizing or reducing agents may be external redox-active substances, i.e. they are not covalently joined with but in contact with the catalytically redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, the substrates specific to the catalytically redox-active moiety, in particular, being able to act as external oxidizing or reducing agents. In addition, an external oxidizing or reducing agent also may be covalently joined with the nucleic acid oligomer, the oxidizing or reducing agent being covalently attached to the nucleic acid oligomer at a site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety, preferably at the oligonucleotide end opposite the modification with the catalytically redox-active moiety, near the conductive surface. In particular, also the conductive surface (electrode) may act as the external oxidizing or reducing agent. The ability to act as an electron donor or acceptor is relative, i.e. a molecule that acts as an electron donor toward another molecule, directly or under the influence of certain external conditions, may also act as an electron acceptor toward that molecule under differing experimental conditions, or toward a third molecule under the same or differing experimental conditions.

electron acceptor

In the context of the present invention, the term "electron acceptor" refers to a component of a catalytically redox-active moiety. An electron acceptor is a molecule that can take up an electron from an electron donor, directly or under the influence of certain external conditions. For example, one such external condition is the

oxidation or reduction of the electron donor or acceptor of the catalytically redox-active moiety by an external oxidizing or reducing agent, so for example the transfer of an electron to the electron donor by a reducing agent, or the release of an electron by the electron acceptor to an oxidizing agent. These oxidizing or reducing agents may be external redox-active substances, i.e. they are not covalently joined with but in contact with the catalytically redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, the substrates specific to the catalytically redox-active moiety, in particular, being able to act as external oxidizing or reducing agents. In addition, an external oxidizing or reducing agent can also be covalently joined with the nucleic acid oligomer, the oxidizing or reducing agent being covalently attached to the nucleic acid oligomer at a site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the catalytically redox-active moiety, preferably at the oligonucleotide end opposite the modification with the redox-active moiety, near the conductive surface. In particular, also the conductive surface (electrode) may act as the external oxidizing or reducing agent. The ability to act as an electron acceptor or donor is relative, i.e. a molecule that acts as an electron acceptor toward another molecule, directly or under the influence of certain external conditions, may also act as an electron donor toward that molecule under differing experimental conditions, or toward a third molecule under the same or differing experimental conditions.

oxidizing agent

A chemical compound (chemical substance) that oxidizes another chemical compound (chemical substance, electron donor, electron acceptor) by taking up electrons from this other chemical compound (chemical substance, electron donor, electron acceptor). An oxidizing

	agent behaves analogously to an electron acceptor, but is used in the context of the present invention to denote an external electron acceptor not directly belonging to the catalytically redox-active moiety. In this context, "not directly" means that the oxidizing agent is either a substrate specific to the catalytically redox-active moiety, or a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer. In addition, the oxidizing agent may be covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the catalytically redox-active moiety. In particular, the electrode also may represent the oxidizing agent.
reducing agent	A chemical compound (chemical substance) that, by giving up electrons to another chemical compound (chemical substance, electron donor, electron acceptor), reduces this other chemical compound (chemical substance, electron donor, electron acceptor). A reducing agent behaves analogously to an electron donor but is used in the context of the present invention to denote an external electron donor not directly belonging to the catalytically redox-active moiety. In this context, "not directly" means that the reducing agent is either a substrate specific to the catalytically redox-active moiety, or a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, or that the reducing agent is covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety. In particular, the electrode also may represent the reducing agent.
redox-active	Redox-active refers to the property of a redox-active moiety of giving up electrons to a suitable oxidizing agent or taking up

	electrons from a suitable reducing agent under certain external conditions, or the property of a redox-active substance of giving up electrons to a suitable electron acceptor or taking up electrons from a suitable electron donor under certain external conditions.
analyte	Equivalent to a substrate.
substrate	A free oxidizing or reducing agent not covalently joined with but in contact with the catalytically redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution applied to the modified conductive surface, the substrate being able to be for example a charged or uncharged molecule, any salt, an ion, or a redox-active protein or enzyme (oxido-reductase). The substrate is characterized in that it is recognized by the catalytically redox-active moiety due to the formation of specific interactions between the substrate and the catalytically redox-active moiety and can reduce the donor (or oxidize the acceptor) of the catalytically redox-active moiety, the catalytic activity of the catalytically redox-active moiety accelerating (catalyzing) this redox reaction of the substrate to the product.
catalytic activity	The catalytic activity of the catalytically redox-active moiety has an accelerating effect on the specific reaction between the moiety and the affiliated substrate and thus allows a reaction course in which the catalytic activity of the moiety is imperceptible or nonexistent. This catalytic activity of the redox-active moiety is achieved by stabilizing the relevant transitional state, i.e. the highest-energy species, in the reaction course between the catalytically redox-active moiety and the affiliated substrate.
electrocatalytic activity	The electrocatalytic activity of the catalytically redox-active moiety is closely related to the catalytic activity of the moiety. The presence of the catalytically redox-active moiety and its integration in the course of the electrode reaction of the substrate to the product (the course

of the entire electrochemical redox reaction between an electrode and the substrate, i.e. the electrode giving up electrons to the substrate or the substrate giving up electrons to the electrode, to the intermediate steps of the redox reaction between the substrate and the catalytically redox-active moiety and the redox reaction between the redox-active moiety and the electrode) accelerates the electrochemical conversion of the substrate at the electrode. The electrocatalytic activity of a catalytically redox-active moiety immobilized at an electrode reduces the activation energy of the electrode reaction of the substrate to the product (the energy of the highest-energy state for the course of the conversion of the substrate to the product at the electrode) and thus causes a shift in the electrode potential required for the electrode reaction of the substrate to the product, in the direction of the equilibrium potential for this electrode reaction. Decreasing the activation potential causes a reduction of the overpotential required for an electrode reaction, and thus an increase in the flow of electrons between the electrode and the substrate at a specific electrode potential that is suitable for the electrode reaction (this increase is generally referred to as "catalytic current"). An important result of the electrocatalytic activity is thus that the electrochemical conversion of the substrate to the product can be carried out in the presence and with the participation of the catalytically redox-active moiety at an electrode potential at which, in the absence of the catalytically redox-active moiety, very little or no current flows.

specificity of the catalytically redox-active moiety

The catalytically redox-active moiety acts specifically both with a view to the substrate that interacts with the catalytically redox-active moiety and with a view to the reaction carried out with the relevant substrate. In the context of the present invention, redox reactions are the preferred reactions between the catalytically redox-active moiety and the substrate.

initiation process	Given appropriately chosen external conditions, the catalytically redox-active moiety exhibits its redox activity, in other words its property of for example giving up electrons to a suitable oxidizing agent or taking up electrons from a suitable reducing agent, only subsequent to an initiation process. Such an initiation process may be the addition of substrate with its property of transferring charge to the catalytically redox-active moiety: thus, the reductive property of a catalytically redox-active moiety becomes possible only through the transfer of the electron(s) from the substrate to the/an electron donor "D," either in the presence of an oxidizing agent that can oxidize D^- but not D, or because, subsequent to successive charge transfer within the catalytically redox-active moiety, the electron is transferred from D^- to an acceptor "A" (directly or via multiple electron transfer steps to intermediate electron acceptors) and an oxidizing agent is present that takes up electrons only from this reduced acceptor "A^-" of the catalytically redox-active moiety, but not from A. In particular, this oxidizing agent may also be an electrode, for example if the electrode is set to a potential at which D^- but not D (or A^- but not A) is oxidized. On the other hand, the oxidative property of a catalytically redox-active moiety becomes possible only through the transfer of the electron(s) from an electron donor "D" to the substrate, either in the presence of a reducing agent that can reduce D^+ but not D, or because, subsequent to successive charge transfer within the catalytically redox-active moiety, an electron is transferred from an acceptor "A" to the oxidized donor D^+ (directly or via multiple electron transfer steps from intermediate electron donors) and a reducing agent is present that gives up electrons only to this oxidized acceptor "A^+" of the catalytically redox-active moiety, but not to A. In particular, this reducing agent may also be an electrode, for example if the

	electrode is set to a potential at which D^+ but not D (or A^+ but not A) is reduced.
redox-active protein/enzyme	Usually consists of what is known as 'apoprotein,' the preferred macromolecule(s) of the present invention, and cofactors, the electron donor(s) and/or electron acceptor(s) within the meaning of the present invention. The redox activity of the redox-active protein/enzyme is triggered by a free redox-active substance (the specific substrate).
oxidase	A class of redox-active enzymes that catalyze the oxidation of the substrate specific to the relevant oxidase.
reductase	A class of redox-active enzymes that catalyze the reduction of the substrate specific to the relevant reductase.
oxido-reductases	Generic term for oxidases and reductases.
GOx	Glucose oxidase (β-D-glucose: oxygen 1-oxido-reductase, EC 1.1.3.4). An example of a redox-active protein/enzyme. The protein/enzyme is an enzyme comprising apoprotein and FAD as a cofactor, cf. ~~Structure 1~~**Fig. 7** and Formula 1. The GOx is present as a homodimeric enzyme (Hecht et al., J. Mol. Biol. 229 (1993), pp. 153-172).
ADH	Alcohol dehydrogenase (EC 1.1.1.1). An example of a redox-active protein/enzyme. The protein/enzyme is an enzyme comprising apoprotein comprising three protein subunits and PQQ, heme, and a heme dimer as cofactors (Amayama et al., Methods Enzymol. 89 (1982) 450-457).
FDH	Fructose dehydrogenase (EC 1.1.99.11). An example of a redox-active protein/enzyme. The protein/enzyme is an enzyme comprising apoprotein and PQQ as (one of) the cofactor(s). The structure of this enzyme is unknown.
LDH	Lactate dehydrogenase (EC 1.1.1.27), an enzyme comprising apoprotein, FMN, and heme.
FAD	flavin adenine dinucleotide, cf. Formula 1
NAD^+	nicotinamide adenine dinucleotide, cf. Formula 2
PQQ	Pyrroloquinoline quinone; corresponds to 4,5-dihydro-4,5-dioxo-

	1H-pyrrolo-[2,3-f]-quinoline-2,7,9-tricarboxylic acid, cf. Formula 3 ($R_1 = R_3 = R_5 = CO_2H$; $R_2 = R_4 = H$) or a derivative thereof (Formula 3).
Heme	Iron-protoporphyrin IX, Formula 4 with $R_2 = R_5 = R_8 = R_{10} = H$; $R_4 = R_6 = R_9 = R_{12} = CH_3$; $R_1 = R_3 = CH_2\text{-}CH_2\text{-}CO_2^-$; $R_7 = R_9 = CH{=}CH_2$, or a derivative of iron-protoporphyrin (Formula 4).
N^6-(2-aminoethyl)-FAD	modified flavin adenine dinucleotide, cf. Formula 5
N^6-(2-aminoethyl)-NAD^+	modified nicotinamide adenine dinucleotide, cf. Formula 6
EDTA	ethylenediamine tetraacetate (sodium salt)
sulfo-NHS	N-hydroxysulfosuccinimide
EDC	(3-dimethylaminopropyl)-carbodiimide
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
Tris	trishydroxymethylamino methane
alkyl	The term "alkyl" refers to a saturated hydrocarbon group that is straight-chain or branched (e.g. ethyl, 2,5-dimethylhexyl, or isopropyl, etc.). When "alkyl" is used to indicate a linker or spacer, the term refers to a group having two available valences for covalent linkage (e.g. $-CH_2CH_2-$, $-CH_2C(CH_3)_2CH_2CH_2C(CH_3)_2CH_2-$ or $-CH_2CH_2CH_2-$, etc.). Alkyl groups preferred as substituents or side chains R are those having a chain length of 1 - 30 (the longest continuous chain of atoms covalently bound to one another). Alkyl groups preferred as linkers or spacers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length here representing the shortest continuous link between the structures joined via the linker or spacer, in other words between the two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule.
alkenyl	Alkyl groups in which one or more of the C-C single bonds are replaced by C=C double bonds.
alkynyl	Alkyl or alkenyl groups in which one or more of the C-C single or

	C=C double bonds are replaced by C≡C triple bonds.
heteroalkyl	Alkyl groups in which one or more of the C-H bonds or C-C single bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkenyl	Alkenyl groups in which one or more C-H bonds, C-C single or C=C double bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkynyl	Alkynyl groups in which one or more of the C-H bonds, C-C single, C=C double or C≡C triple bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
linker	A molecular link between two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule. Linkers can usually be purchased in the form of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl chains, the chain being derivatized in two places with (identical or different) reactive groups. These groups form a covalent chemical bond in simple/known chemical reactions with the appropriate reaction partners. The reactive groups may also be photoactivatable, i.e. the reactive groups are activated only by light of a specific or any given wavelength. Preferred linkers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length here representing the shortest continuous link between the structures to be joined, in other words between the two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule.
spacer	A linker that is covalently attached via the reactive groups to one or both of the structures to be joined (see linker). Preferred spacers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length representing the shortest continuous link between the structures to be joined.
(n x HS-spacer)-oligo	A nucleic acid oligomer to which n thiol functions are each attached via a spacer, each spacer being able to have a different chain length (the shortest continuous link between the thiol

	function and the nucleic acid oligomer), especially any chain length between 1 and 14. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification, and "n" is any integer, especially a number between 1 and 20.
(n x R-S-S-spacer)-oligo	A nucleic acid oligomer to which n disulfide functions are each attached via a spacer, the disulfide function being saturated by any residue R. Each spacer for attaching the disulfide function to the nucleic acid oligomer may have a different chain length (shortest continuous link between the disulfide function and the nucleic acid oligomer), especially any chain length between 1 and 14. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification. The variable n is any integer, especially a number between 1 and 20.
oligo-spacer-S-S-spacer-oligo	Two identical or different nucleic acid oligomers that are joined with each other via a disulfide bridge, the disulfide bridge being attached to the nucleic acid oligomers via any two spacers, the two spacers being able to have differing chain lengths (the shortest continuous link between the disulfide bridge and the respective nucleic acid oligomer), especially any chain length between 1 and 14, and these spacers, in turn, being able to be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification.
mica	Muscovite lamina, a support material for the application of thin films.
Au-S-$(CH_2)_2$-ss-oligo-spacer-PQQ-FAD(GOx)	Gold film on mica having a covalently applied monolayer comprising derivatized 12-bp single-strand DNA oligonucleotide (sequence: TAGTCGGAAGCA). Here, the oligonucleotide's terminal phosphate group at the 3'-end is esterified with $(HO-(CH_2)_2-S)_2$ to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH, the S-S bond being homolytically cleaved and producing one Au-S-R bond each. The terminal thymine base at the 5'-end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2, this residue, in turn, being joined

	via its free amino group with the carboxylic-acid group of the PQQ by amidation. To a further carboxylic-acid group of this PQQ is bound by amidation FAD that was previously modified in such a way that it has a reactive amino group at its disposal, for example by forming N^6-(2-aminoethyl)-FAD (Bückmann et al., 1991, European Patent 0.247.537.B1). Thereafter, the FAD is reconstituted with the apoprotein of the GOx such that a nucleic acid oligomer results that is covalently attached to the surface and, in addition – via PQQ as a covalently attached bridge molecule –, is covalently modified with the complete GOx moiety.
Au-S-$(CH_2)_2$-ds-oligo-spacer-PQQ-FAD(GOx)	*Au-S-$(CH_2)_2$-ss-oligo-spacer-PQQ-FAD(GOx)* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence: TAGTCGGAAGCA).
Au-S-$(CH_2)_2$-ss-oligo-spacer-PQQ-NAD^+-LDH	Gold film on mica having a covalently applied monolayer comprising derivatized 12-bp single-strand DNA oligonucleotide (sequence: TAGTCGGAAGCA). Here, the oligonucleotide's terminal phosphate group at the 3'-end is esterified with $(HO-(CH_2)_2-S)_2$ to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH, the S-S bond being homolytically cleaved and producing one Au-S-R bond each. The terminal thymine base at the 5'-end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2, this residue, in turn, being joined via its free amino group with the carboxylic-acid group of the PQQ by amidation. To a further carboxylic-acid group of this PQQ is bound by amidation NAD^+ that was previously modified in such a way that it has a reactive amino group at its disposal, for example by forming N^6-(2-aminoethyl)-NAD^+ (Bückmann et al., 1991, European Patent 0.247.537.B1). The complete LDH is associated at this terminal NAD^+.
Au-S-$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-LDH	*Au-S-$(CH_2)_2$-ss-oligo-spacer-PQQ-NAD^+-ADH* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence: TAGTCGGAAGCA).
E	The electrode potential on the working electrode.
E^{eq}	The equilibrium potential of an electrode reaction.
^{0}	The "zero current" potential of an electrode reaction, the

	potential that does not supply total current (the sum of the oxidative and reductive current) for a specific electrode reaction.
h	The overpotential of an electrode reaction.
Electrode reaction	The redox reaction between a redox-active substance and an electrode (taking up electrons from the electrode by the redox-active substance or giving up electrons from the redox-active substance to the electrode).
E_{Ox}	The potential at maximum current of the oxidation of a reversible electrooxidation or electroreduction.
E_{Red}	The potential at maximum current of the reduction of a reversible electrooxidation or electroreduction.
I	current density (current per cm^2 of electrode surface)
cyclic voltammetry	Recording a current-voltage curve. Here, the potential of a stationary working electrode is changed linearly as a function of time, starting at a potential at which no electrooxidation or electroreduction occurs, up to a potential at which a species that is dissolved or adsorbed on the electrode is oxidized or reduced (i.e. a current flows). After running through the oxidation or reduction operation, which produces in the current-voltage curve an initially increasing current and, after reaching a maximum, a gradually decreasing current, the direction of the potential feed is reversed. The behavior of the products of electrooxidation or electroreduction is then recorded in a reverse run.
amperometry	Recording a current-time curve. Here, the potential of a stationary working electrode is set, for example by a potential jump, to a potential at which the electrooxidation or electroreduction of a dissolved or adsorbed species occurs, and the flowing current is recorded as a function of time.
potentiometry	Recording an electrode voltage course as a function of, for example, substrate consumption. Here, the potential of a stationary working electrode is set, for example, to the "zero current" potential E^0 of the substrate. When the substrate is consumed by the catalytically redox-active moiety (in the case of hybridization), the "zero current"

potential E^0 changes in the direction of the equilibrium potential E^{eq}. Thus, recording the potential as a function of time (~ substrate consumption) provides information on the hybridization state.

[021] The present invention is directed to a nucleic acid oligomer that is modified by chemically binding a catalytically redox-active moiety. After giving up an electron to an external oxidizing agent (substrate), the catalytically redox-active moiety may be reduced by an external reducing agent, for example an electrode, or after taking up an electron from an external reducing agent (substrate), be oxidized by an external oxidizing agent, for example an electrode. **According to the present invention, the catalytically redoxaktive moiety is selected from the group consisting of native or modified alcohol dehydrogenase, native or modified fructose dehydrogenase, native or modified lactate dehydrogenase, and native or modified peroxidase.**

[022] In the context of the present invention, a compound comprising at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine (e.g. cytosine, thymine, or uracil) or purine bases (e.g. adenine or guanine), preferably a DNA, RNA, or PNA fragment, is used as the nucleic acid oligomer. In the present invention, the term "nucleic acid" refers to any backbone of the covalently-joined pyrimidine or purine bases, such as the sugar-phosphate backbone of DNA, cDNA, or RNA, a peptide backbone of PNA, or analogous backbone structures such as a thiophosphate, a dithiophosphate, or a phosphoramide backbone. An essential feature of a nucleic acid within the meaning of the present invention is that it can sequence-specifically bind naturally occurring DNA or RNA. The terms "(probe) oligonucleotide", "nucleic acid", and "oligomer" are used as alternatives to the term "nucleic acid oligomer".

[023] In the context of the present invention, the term "electron acceptor" or "electron-acceptor molecule" and the term "electron donor" or "electron-donor molecule" refer to a component (a redox-active center, cofactor, or prosthetic group) of a catalytically redox-active moiety.

[024] In the context of the present invention, a moiety referred to using the generic term "catalytically redox-active moiety" usually consists of one or more redox-active centers (cofactors, prosthetic groups), referred to in the following as electron donors or electron acceptors, and one or more macromolecules binding these redox-active centers. ~~Thus, in its form that is relevant to the present invention~~ **The catalytically redox-active moiety according to the present inventon is**

selected form the group consisting of native or modified alcohol dehydrogenase, native or modified frustose dehydrogenase, native or modified lactate dehydrogensase, and native or modified peroxidases.

[025] The catalytically redox-active moiety contains one or more electron-donor molecules and/or one or more electron-acceptor molecules, this (these) electron-donor molecule(s) and/or this (these) electron-acceptor molecule(s) being bound to one or more macromolecules or being embedded in this (these) macromolecule(s). Electron donor(s) and/or electron acceptor(s) may be joined with one another via one or more covalent or ionic bonds, via hydrogen bonds, van der Waals bridges, via π-π-interaction, or via coordination by means of electron-pair donation and acceptance, the covalent links being able to be direct or indirect links (e.g. via a spacer, but not via a nucleic acid oligomer). In addition, the electron donor(s) and/or electron acceptor(s) may be joined with the macromolecule(s) via covalent attachment to the macromolecule(s), via encapsulation in suitable molecular cavities (binding pockets) of the macromolecule(s), via ionic bonds, hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance between the macromolecule(s) and the electron-donor molecule(s) and/or the electron-acceptor molecule(s). If multiple macromolecules are components of the catalytically redox-active moiety, the binding of the macromolecules to one another may likewise take place covalently, ionically, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance. In the minimum case, a catalytically redox-active moiety may also consist of only one macromolecule, the macromolecule, in its form that is relevant to the present invention, then also acting as the electron donor or acceptor. It may also consist of only one electron donor or acceptor. In addition, the catalytically redox-active moiety may also be formed through spontaneous congregation of the components in solution (in situ).

[026] The aforementioned donor and/or acceptor molecules form, together with the macromolecules, a catalytically redox-active moiety, i.e., they are bound to one another directly or via further molecular moieties. The sole restriction on the molecules or molecular moieties joining the components of the catalytically redox-active moiety is the exclusion of nucleic acid oligomers. According to the present invention, the catalytically redox-active moiety is bound to the probe oligonucleotide

active moiety, the invention represents a microelectrode-targetable method of qualitatively and quantitatively detecting, in parallel, redox-active substances, the relevant substrate of the various catalytically redox-active moieties of the electrodes within an electrode array.

Binding a Catalytically Redox-Active Moiety to a Nucleic Acid Oligomer

[040] A prerequisite for the method according to the present invention is the binding of a catalytically redox-active moiety to a nucleic acid oligomer. The catalytically redox-active moiety may be ~~for example any redox-active protein/enzyme from the group of oxidases or reductases, protein-engineering or gene-mutation-modified proteins/enzymes from this group of oxidases or reductases, or an artificially produced moiety comprising one or more redox-active centers (electron donor or acceptor), or an artificially produced moiety comprising one or more redox-active centers (electron donor or acceptor) and one or more macromolecules binding these redox-active centers.~~

~~[037] The following are some examples of a catalytically redox-active moiety:~~

~~(i) Redox-active proteins/enzymes, such as the oxido-reductases, some of which are~~**native or modified alcohol dehydrogenase, native or modified fructose dehydrogenase, native or modified lactate dehydrogenase, and native or modified peroxidase.**

[041] Catalytically redox-active moieties are:

(i) The oxido-reductases compiled in Table 1 below. The covalent attachment of the catalytically redox-active moiety (the redox-active protein/enzyme) occurs, in the context of the present invention, preferably via a covalent attachment of the cofactor with subsequent reconstitution of the apoprotein to the cofactor attached to the nucleic acid oligomer. In catalytically redox-active moieties (redox-active proteins/enzymes) having multiple cofactors, one of the cofactors (appears in bold in Table 1 below) is covalently attached to the nucleic acid oligomer, and the catalytically redox-active moiety is completed by reconstituting it with the remaining cofactors and the apoprotein.

Table 1: ~~A selection of some redox-active~~ **Redox-active** enyzmes (oxido-reductases) as ~~examples of~~ catalytically redox-active moieties.

Enzyme	Cofactor	Substrate	Catalyzed enzyme reaction
~~Glucose oxidase~~	~~FAD~~	~~Glucose~~	~~Glucose + FAD → gluconic acid + $FADH_2$~~
Alcohol dehydrogenase	**PQQ**, heme, heme dimer	Ethanol	Ethanol + PQQ → acetaldehyde + $PQQH_2$
Fructose dehydrogenase	**PQQ**, heme, ...	Fructose	D-fructose + PQQ → 5-keto-d-fructose + $PQQH_2$
Lactate dehydrogenase	**FMN**, heme	Lactate	Lactate + FMN → pyruvate + $FMNH_2$
Peroxidases (e.g. horseradish peroxidase, lactoperoxidase, cytochrom c peroxidase, fungal peroxidase, etc.)	Heme		

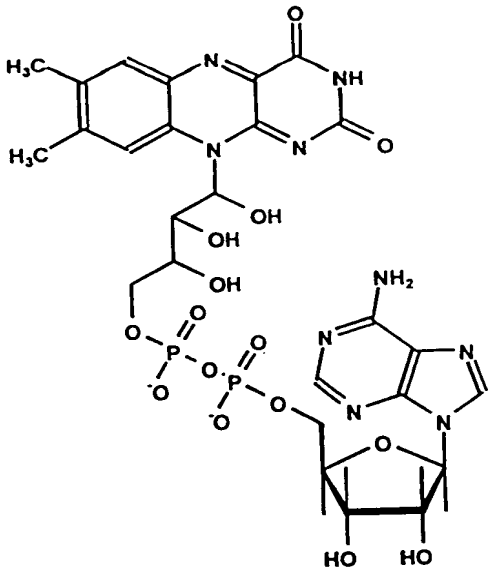
[042] (ii) Modified redox-active proteins/enzymes as described under (i) that were modified through protein engineering or gene mutation and that continue to possess catalytic or electrocatalytic activity.

~~[039] (iii) Artificially produced catalytically redox-active moieties comprising electron donor(s) and/or electron acceptors and macromolecules possessing catalytic or electrocatalytic activity.~~

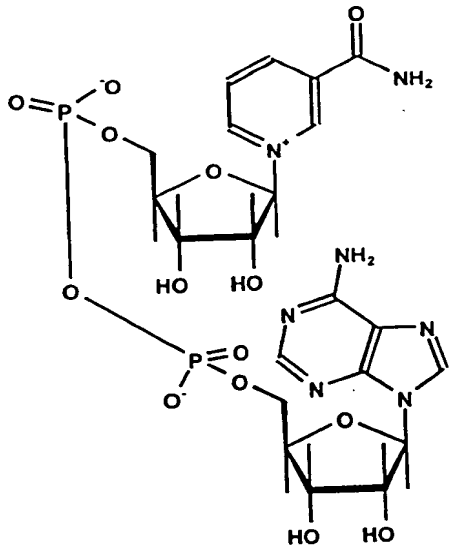
[043] (~~iv~~iii) NAD^+-dependent enzymes such as lactate dehydrogenase (LDH, EC 1.1.1.27) or alcohol dehydrogenase (ADH, EC 1.1.1.1). If NAD^+-dependent enzymes are used, the catalytically redox-active moiety (e.g. LDH or ADH) may be attached to the nucleic acid oligomer by covalently binding (modified) NAD^+ to the nucleic acid oligomer, directly or via a spacer (Example 3), and then associating the NAD^+-dependent enzyme with the (modified) NAD^+ through non-covalent interaction.

[044] ~~*Structure 1*: Monomer of glucose oxidase (GOx). The apoprotein consists of a-helical and b-pleated sheet domains; the coenzyme flavin adenine dinucleotide (FAD) is drawn in the form of the space-filling shallot model. The structure of the FAD is shown in Formula 1. In its native form, the GOx is present as a homodimer.~~ **Fig. 7 shows the monomer of the glucose oxidase (GOx). The apoprotein consists of α-helicals and β-pleated sheet domains; the coenzyme flavin adenine dinucleotide (FAD) is drawn in the form of the space-filing shallot model. The- structure of the FAD is shown in Formula 1. In its native form, the GOx is present as a homodimer.**

[045]



Formula 1



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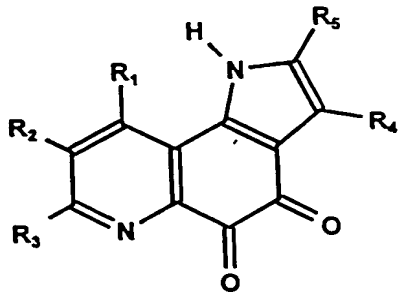

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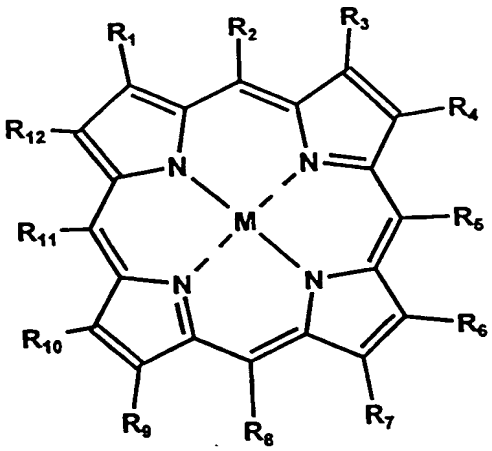
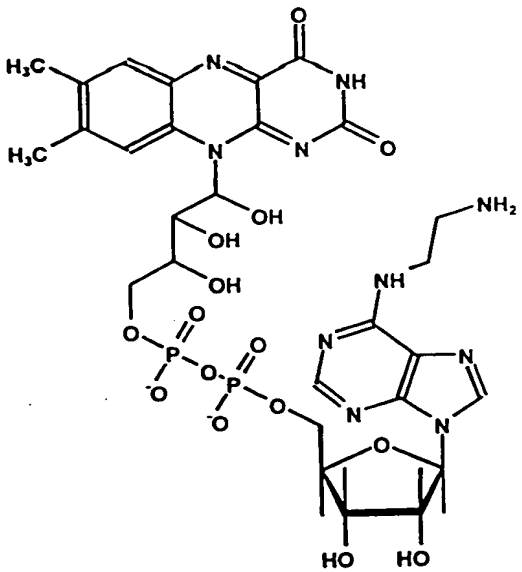
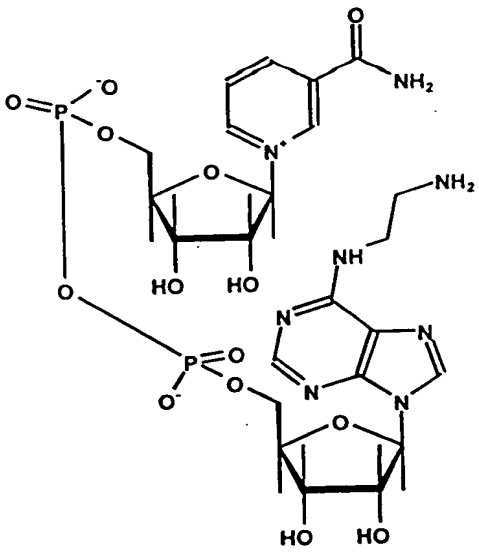


Formula 4

M = 2H, Mg, Zn, Cu, Ni, Pd, Co, Cd, Mn, Fe(II), Fe(III), Sn, Pt, etc.; R_1 to R_{12} are, independently of one another, H or any alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituents.



Formula 5



Formula 6

[062] Furthermore, parallel detection of hybridization events may also be achieved either by using various catalytically redox-active moieties for the individual electrodes of the array when forming the various functionalized electrodes of an electrode array or by using a continuously conductive surface to form the functionalized electrodes and by achieving the differentiability of molecular structures on a specific area having an identical electrode structure (to that of a specific test site) within the entire system (of the complete oligomer chip) by using, for the individual test sites, various catalytically redox-active moieties that can be addressed by selectively adding the relevant specific substrate. In the latter variant, the electrochemical response of the entire oligomer chip is detected as a result of the continuous conductive surface; addressing and reading out the electrochemical response of individual test sites occurs by selectively adding the relevant specific substrate for this test site.

[063] The modification of the probe nucleic acid oligomers with a catalytically redox-active moiety may take place completely or in components of the catalytically redox-active moiety, either before or after the oligonucleotide probe is bound to the conductive surface. The various combination possibilities of the individual steps (reaction sequences) are demonstrated in the section "Manner of Executing the Invention" with the aid of Fig. 2 using the example of a catalytically redox-active moiety bound to an electrode via a probe oligonucleotide.

[064] Regardless of the respective reaction sequence, a surface hybrid having the general structure elec-spacer-ss-oligo-spacer-moiety results, "moiety" representing the catalytically redox-active moiety. The bridges may, of course, also be produced without spacers or with only one spacer (elec-ss-oligo-spacer-moiety or elec-spacer-ss-oligo-moiety). In the example in Fig. 2, the moiety is the glucose oxidase (GOx), a redox-active enzyme consisting of apoprotein and cofactor. In the example in Figs. 2, 3a, and 4, the GOx, via its cofactor flavin adenine dinucleotide (FAD) in what is known as the FAD protein binding pocket of the GOx, is covalently joined with the nucleic acid oligomer. The GOx forms a 1:1 complex with the cofactor FAD, the GOx occurring in its natural form as a homodimer, but also exhibiting catalytic activity as a monomer, its form that is relevant to the present invention. In the example in Figs. 5a and 6, the moiety is lactate dehydrogenase, a NAD^+-dependent enzyme, that associates with the (modified) NAD^+ that is covalently bound to the probe oligonucleotide via non-covalent interaction.

[065] The electrochemical communication between the (conductive) surface and the catalytically redox-active moiety ("moiety") bridged via a single-strand oligonucleotide having the general structure elec-spacer-ss-oligo-spacer-moiety is weak or nonexistent.

[066] In a next step, the test sites are brought into contact with the nucleic acid oligomer solution to be examined (target). This leads to hybridization only if the solution contains nucleic acid oligomer strands that are complementary to the probe nucleic acid oligomers bound to the conductive surface, or complementary in at least wide areas. Hybridization between the probe and target nucleic acid oligomers leads to increased conductivity between the surface and the catalytically redox-active moiety, since the latter is now bridged via the nucleic acid oligomer consisting of a double-strand. _Figs. 3**3a-3c** illustrates this schematically using elec-spacer-ss-oligo-spacer-FAD(GOx) as an example. In Fig. 4, the sequence of the electron transfer steps in elec-spacer-ds-oligo-spacer-FAD(GOx) is shown in detail, while Fig. 5**a** schematically illustrates the example elec-spacer-ss-oligo-spacer-PQQ-NAD^+-LDH and Fig. 6 shows in detail the sequence of the electron transfer steps in elec-spacer-ds-oligo-NAD^+-LDH.

[067] As a result of the hybridization of the probe nucleic acid oligomer and the nucleic acid oligomer strand (target) that is complementary thereto, the electrical communication between the (conductive) surface and the catalytically redox-active moiety changes. Thus, a sequence-specific hybridization event can be detected by electrochemical methods such as cyclic voltammetry, amperometry, potentiometry, or conductivity measurements.

[068] In cyclic voltammetry, the potential of a stationary working electrode is changed linearly as a function of time. Starting at a potential at which no electrooxidation or electroreduction occurs, the potential is changed until the redox-active substance is oxidized or reduced (i.e., a current flows). After running through the oxidation or reduction operation, which produces in the current-voltage curve an initially increasing current, then a maximum current (peak), and finally a gradually decreasing current, the direction of the potential feed is reversed. The behavior of the products of electrooxidation or electroreduction is then recorded in reverse.

[069] An alternative electrical detection method, amperometry, is made possible by applying a suitable constant electrode potential such that the catalytically redox-

active moiety may be electrooxidized (electroreduced), but the rereduction (reoxidation) of the catalytically redox-active moiety to its original state takes place, not by changing the electrode potential as in cyclic voltammetry, but rather by adding a suitable reducing agent (oxidizing agent), the "redox-active substance," to the target solution, thereby closing the current circuit of the entire system. As long as such a reducing agent (oxidizing agent) is present, or as long as the consumed reducing agent (oxidizing agent) is rereduced (reoxidized) at the counter electrode, a current flows that can be amperometrically detected and that is proportional to the number of hybridization events.

[070] This principle of amperometric detection will be explained in greater detail using the example of glucose oxidase (cf. also Figs. ~~3~~**3a-3c** and 4). The probe oligonucleotide having one end covalently attached to the electrode can be functionalized at the other, free end with the complete glucose oxidase enzymatic moiety, for example by covalently attaching the flavin adenine dinucleotide (FAD) cofactor of the enzyme to the probe oligonucleotide and subsequently reconstituting it with the glucose oxidase apoprotein (GOx). The resultant surface hybrid having the general structure elec-spacer-ss-oligo-spacer-FAD(GOx) exhibits little or no conductivity between the electrode and the FAD. Hybridization with the "ss-oligo"-complementary target oligonucleotide significantly increases the conductivity. Upon adding the glucose substrate to the target oligonucleotide solution, the FAD of the glucose oxidase (FAD(GOx)) is reduced to $FADH_2$ of the glucose oxidase ($FADH_2$(GOx)), glucose being oxidized to gluconic acid. If a suitable external potential is then applied to the electrode such that electrons from $FADH_2$(GOx) are given up to the electrode via the hybridized oligonucleotide, and $FADH_2$(GOx) is thus reoxidized to FAD(GOx) (but neither glucose nor gluconic acid can be electrooxidized or electroreduced at this potential), a current will flow in the elec-spacer-ds-oligo-spacer-FAD(GOx) system as long as FAD(GOx) is reduced by free glucose, i.e. until all of the glucose is consumed or, in the event that a potential at which gluconic acid can be reduced to glucose is applied to the counter electrode, as long as gluconic acid is reduced at the counter electrode. This current can be detected amperometrically and is proportional to the number of hybridization events.

[071] In potentiometric detection of hybridization events, the course of the electrode potential is recorded as a function of, for example, substrate

consumption. Here, for example, the potential of a stationary working electrode is set to the substrate's "zero current" potential E^0. When the substrate is consumed by the catalytically redox-active moiety (in the event of hybridization), the "zero current" potential E^0 changes in the direction of the equilibrium potential E^{eq}. Thus, recording the potential as a function of time (~ substrate consumption) provides information on the hybridization state.

[072] BRIEF DESCRIPTION OF THE DRAWINGS

[073] The invention will be explained in greater detail below by reference to exemplary embodiments in association with the drawings, wherein:

[074] Fig. 1 Shows a schematic diagram of oligonucleotide sequencing by hybridization on a chip;

[075] Fig. 2 Shows various reaction sequences for producing the surface hybrid elec-spacer-ss-oligo-spacer-PQQ-FAD(GOx). The catalytically redox-active moiety in this surface hybrid is the glucose oxidase (GOx) consisting of the apoprotein and the flavin adenine dinucleotide (FAD) cofactor. The GOx, via its cofactor FAD, is covalently joined via PQQ and a spacer with the oligonucleotide;

[076] Figs. ~~3~~**3a-3c** Shows a schematic diagram of the amperometric measurement method using the example of the surface hybrid elec-spacer-ss-oligo-spacer-PQQ-FAD(GOx) in Fig. 2 (Inj: addition (injection) of the glucose substrate);

[077] Fig. 4 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-FAD(GOx) of Fig. ~~3~~**3a** having gold as the surface material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH-PQQ-NH-CH_2-CH_2- as the spacer between the cofactor FAD and the oligonucleotide, as well as a diagram of the sequence of the substrate-induced electron transfer steps. The apoprotein of the GOx is indicated only as a shell (solid line) (cf. ~~Structure 1~~**Fig. 7**). The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail;

[078] Fig. 5 Shows a schematic diagram of the amperometric measurement method using the example of the surface hybrid elec-spacer-ss-oligo-spacer-PQQ-NAD^+-LDH (Inj: injection of the lactate substrate); ~~and~~

[079] Fig. 6 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-LDH of Fig. ~~3~~**3a** having gold as the surface

material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH-PQQ-NH-CH_2-CH_2- as the spacer between the NAD^+ and the oligonucleotide to which ADH is associated, as well as a diagram of the sequence of the substrate-induced electron transfer steps. The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail.

[080] **Fig. 7 The monomer of the glucose oxidase (GOx). The apoprotein consists of α-helicals and β-pleated sheet domains, the coenzyme flavin adenine dinucleotide (FAD) is drawn in the form of the space-filling shallot model. The structure of the FAD is shown in Formula 1. In its native form, the GOx is present as a homodimer.**

[081] DETAILED DESCRIPTION OF THE INVENTION

[082] A formation unit of an exemplary test site with hybridized target, Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-FAD(GOx) having the general structure elec-spacer-ds-oligo-spacer-moiety, is illustrated in Fig. 4. In the context of the present invention, "formation unit" is understood to mean the smallest repeating unit of a test site or functionalized electrode within the electrode array. In the example in Fig. 4, the surface is a gold electrode. The link between the gold electrode and the probe oligonucleotide was formed with the linker (HO-$(CH_2)_2$-S$)_2$, which was esterified with the terminal phosphate group at the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH and, following homolytic cleavage of the S-S bond at the gold surface, produced one Au-S bond each, with which 2-hydroxy-mercaptoethanol and mercaptoethanol-bridged oligonucleotide was coadsorbed on the surface. The catalytically redox-active moiety in the example in Fig. 4 is the glucose oxidase (GOx), a redox-active enzyme consisting of apoprotein and FAD cofactor(s). In the application example, the GOx, via its FAD cofactor, is covalently joined with the oligonucleotide, free FAD having first been provided with a reactive amino group (see Example 1), then covalently attached to the probe oligonucleotide via this amino group (amidation and dehydration with a carboxylic-acid group of the PQQ bound with another carboxylic-acid group to the terminal amino function of the -CH=CH-CO-NH-CH_2-CH_2-NH_2 linker attached at the C-5 position of the 5' thymine of the probe oligonucleotide), and finally, the apoprotein of the GOx was reconstituted to FAD.

oligonucleotide can first be modified with PQQ and FAD so as then to reconstitute it with the GOx apoprotein and, thereafter, to covalently bind it to the electrode. In the event that, as in the case of the GOx, the catalytically redox-active moiety has a significantly greater diameter than the hybridized ds-oligonucleotide (greater than 30 Å), the covalent attachment of a suitable free monofunctional linker (spacer) to the electrode can be dispensed with; otherwise, the attachment of the structure -spacer-ss-oligo-spacer-PQQ-FAD(GOx) to the electrode occurs in the presence of a suitable free monofunctional linker.

[085] In the example in Fig. 2, the GOx, via its FAD-cofactor is covalently joined with the oligonucleotide. Alternatively, instead of the FAD-cofactor, the apoprotein also can be covalently attached to the probe oligonucleotide, any combinations of the reaction sequences "1," "2," "3," or "4" in Fig. 2 may be applied, as long as they yield the same end product (cf. Fig. 2), and, in any reaction steps, the probe oligonucleotide hybridized with complementary, unmodified (target) oligonucleotide may be used in place of the single-strand probe oligonucleotides. The probe oligonucleotide can also be attached directly, in other words not bridged via a spacer, to both the electrode and the catalytically redox-active moiety, as described under c) in the section "Binding a Nucleic Acid Oligomer to the Conductive Surface" or under a) in the section "Binding a Catalytically redox-active Moiety to a Nucleic Acid Oligomer."

[086] The electrical communication between the conductive surface and the catalytically redox-active moiety bridged via a single-strand oligonucleotide in the general structure elec-spacer-ss-oligo-spacer-moiety is weak or nonexistent. If hybridization occurs between the probe and the target, treating the test site(s) with an oligonucleotide solution to be examined causes increased conductivity between the surface and the catalytically redox-active moiety bridged via a double-strand oligonucleotide. For the formation unit of the test site Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-FAD(GOx) (with 12-bp probe oligonucleotides) used as an example, this is shown schematically in Fig. 3c using amperometric measurements.

[087] By adding glucose, electrons are transferred to the FAD-cofactor of the GOx. If a suitable potential is applied to the electrode to transfer electrons from the reduced FAD (FAD^- or FAD^{2-} or $FADH_2$) to the electrode, current still will not flow in the case of the probe oligonucleotide not hybridized with the target oligonucleotide, since the conductivity of the ss-oligonucleotide in Au-S$(CH_2)_2$-ss-

oligo-spacer-PQQ-FAD(GOx) is very slight or nonexistent. In the hybridized state (Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-FAD(GOx)), however, conductivity is high, electrons can be transferred from the reduced FAD (FAD^- or FAD^{2-} or $FADH_2$) to the electrode (forming FAD). This manifests itself amperometrically in a distinct flow of current between the electrode and the catalytically redox-active moiety (Fig. 3c). It is thus possible to detect the sequence-specific hybridization of the target with the probe oligonucleotides by amperometry. The individual electron transfer steps that are triggered in the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-FAD(GOx) by the substrate are illustrated in Fig. 4. In principle, given suitable external conditions, the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-FAD(GOx) can also be reversed, such that FAD is reduced by the electrode and reduced FAD (FAD^- or FAD^{2-} or $FADH_2$) is oxidized by a suitable substrate in a catalytic reaction.

[088] A further test site or a further functionalized electrode within the electrode array, Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-LDH, having the general structure elec-spacer-ss-oligo-spacer-moiety, is illustrated in Fig. 5a. By adding the ADH substrate lactate, electrons are transferred to the FMN cofactor of the LDH and are passed on from this reduced FMN (FMN^- or FMN^{2-} or $FMNH_2$) to NAD^+, directly or with further cofactors of the LDH participating, and finally, from the reduced NAD^+ (NAD, NAD^-, or NADH) to the electrode. In the case of the probe oligonucleotide not hybridized with the target oligonucleotide, current still will not flow between the reduced NAD^+ (NAD, NAD^-, or NADH) and the electrode, since the conductivity of the ss-oligonucleotide in Au-S$(CH_2)_2$-ss-oligo-spacer-PQQ-NAD^+-LDH is very weak or nonexistent. In the hybridized state (Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-LDH), however, conductivity is high, electrons can be transferred from the reduced NAD^+(NAD, NAD^-, or NADH) to the electrode (forming NAD^+). This manifests itself amperometrically in a distinct flow of current between the electrode and the catalytically redox-active moiety (Fig. 5c). The sequence-specific hybridization of the target with the probe oligonucleotides can thus be detected by amperometry. The individual electron transfer steps that are triggered in the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-ADH by the substrate are illustrated in Fig. 6. In principle, given suitable external conditions, the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-LDH can also be reversed, such that NAD^+ is reduced by the electrode and reduced NAD^+ (NAD, NAD^-, or NADH) is oxidized by a suitable substrate (e.g. acetaldehyde) in a catalytic reaction.